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REVIEW ARTICLE

Transcriptional and post-translational regulation of adiponectin

Meilian LIU* and Feng LIU*†‡[1]

*Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, U.S.A., †Department of Biochemistry, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, U.S.A., and ‡The Barshop Center for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, U.S.A.

Adiponectin is an adipose-tissue-derived hormone with anti-diabetic, anti-atherogenic and anti-inflammatory functions. Adiponectin circulates in the bloodstream in trimeric, hexameric and high-molecular-mass species, and different forms of adiponectin have been found to play distinct roles in the regulation of energy homoeostasis. The serum levels of adiponectin are negatively correlated with obesity and insulin resistance, yet the underlying mechanisms remain elusive. In the present review, we summarize recent progress made on the mechanisms regulating adiponectin gene transcription, multimerization and secretion. We also discuss the potential relevance of these studies to the development of new clinical therapy for insulin resistance, Type 2 diabetes and other obesity-related metabolic disorders.

Key words: adiponectin, Dsb, endoplasmic reticulum stress, inflammation, insulin, peroxisome-proliferator-activated receptor γ (PPARγ), transcription factor.

INTRODUCTION

Adiponectin, a 30-kDa adipokine secreted mainly from adipocytes, plays an important role in the regulation of lipid and glucose metabolism [1–4]. Adiponectin stimulates fatty acid oxidation, suppresses hepatic gluconeogenesis, increases insulin sensitivity and exerts a protective role against chronic inflammation [5–8]. Adiponectin has also been shown to possess anti-atherogenic effects by up-regulating AMPK (AMP-activated protein kinase) signalling [9–11] and to act on the central nervous system to regulate food intake and body weight [12,13]. Thus adiponectin is a promising candidate for the development of drugs to treat obesity, insulin resistance, Type 2 diabetes and other related metabolic diseases.

Adiponectin circulating in serum exists at remarkably high levels ($\sim$10–30 μg/ml or $\sim$0.01 % of plasma proteins) and is present primarily in three species: an LMW (low-molecular-mass) trimer of approx. 67 kDa, a hexamer of $\sim$120 kDa and an HMW (high-molecular-mass) multimer of $>$300 kDa [14–16]. The HMW adiponectin has been shown to possess the most potent insulin-sensitizing activity. Defects in adiponectin multimerization are associated with diabetes and hypoadiponectinaemia [14,16], and increased circulating levels of HMW adiponectin are associated with weight loss [17], improved glucose tolerance [18] and TZD (thiazolidiedione)-mediated increases in insulin sensitivity [19]. The formation of HMW adiponectin in cells is regulated by post-translational modifications such as hydroxylation, glycosylation and disulfide bond formation and impairments in such modification lead to a marked reduction in the intracellular levels of adiponectin and its subsequent secretion [20–22].

The expression levels of adiponectin are stimulated by the TZD class of insulin-sensitizing drugs, suggesting that the adiponectin gene is a target of PPAR (peroxisome-proliferator-activated receptor) γ. In addition to PPARγ, several other transcription factors such as C/EBP (CCAAT/enhancer-binding protein) α, SREBP (sterol-regulatory-element-binding protein)-1c, FoxO1 (forkhead box O1) and Sp1 (specificity protein 1) have also been shown to positively regulate adiponectin gene transcription. The expression of adiponectin is also subjected to negative regulation by insulin resistance-inducing factors such as ROS (reactive oxygen species), TNFα (tumour necrosis factor α) and IL (interleukin)-6. Since dysregulation in adiponectin expression and secretion have links to various metabolic and cardiovascular abnormalities associated with obesity and insulin resistance, understanding the mechanisms regulating adiponectin expression and post-translational modification is thus critical for the development of potential therapeutic treatments for these diseases.

In the present review, we first discuss the mechanisms regulating adiponectin gene transcription, focusing mainly on transcriptional factors known to either positively or negatively regulate the adiponectin transcription process. We then summarize the progress that has been made on post-translational modification of adiponectin, which are critical for the multimerization, stability and secretion of this adipokine. Finally, we review some of the signalling pathways involved in the regulation of adiponectin biosynthesis and secretion.

TRANSCRIPTIONAL REGULATION OF ADIPONECTIN EXPRESSION

Basic structure of the adiponectin gene promoter

The mouse adiponectin gene is a single-copy gene and is mapped to the telomere of chromosome 16, syntenic to the human

Abbreviations used: AP-2β, activating enhancer-binding protein-2β; ATF3, activating transcription factor 3; bHLH, basic helix–loop–helix; C/EBP, CCAAT/enhancer-binding protein; CREB, cAMP-response-element-binding protein; CBP, CREB-binding protein; DsbA-L, disulfide-bond A oxidoreductase-like protein; ER, endoplasmic reticulum; ERK, extracellular-signal-regulated kinase; Ero1, ER membrane-associated oxidoreductase 1; FoxO1, forkhead box O1; GST, glutathione transferase; HMW, high-molecular-mass; Id3, inhibitor of differentiation-3; IGF-1, insulin-like growth factor 1; IGFBP-3, IGF-1-binding protein 3; IL, interleukin; JNK, c-Jun N-terminal kinase; LMW, low-molecular-mass; MAPK, mitogen-activated protein kinase; NFAT, nuclear factor of activated T-cells; PDI, protein disulfide-isomerase; PKA, protein kinase A; PKC, protein kinase C; PPAR, peroxisome-proliferator-activated receptor; PPRE, PPAR-responsive element; RNAi, RNA interference; Sirt1, sirtuin 1; Sp1, specificity protein 1; SRE, sterol-regulatory element; SREBP, sterol-regulatory-element-binding protein; TNFα, tumour necrosis factor α; TZD, thiazolidiedione; WAT, white adipose tissue.

[1] To whom correspondence should be addressed (email liuf@uthscsa.edu).

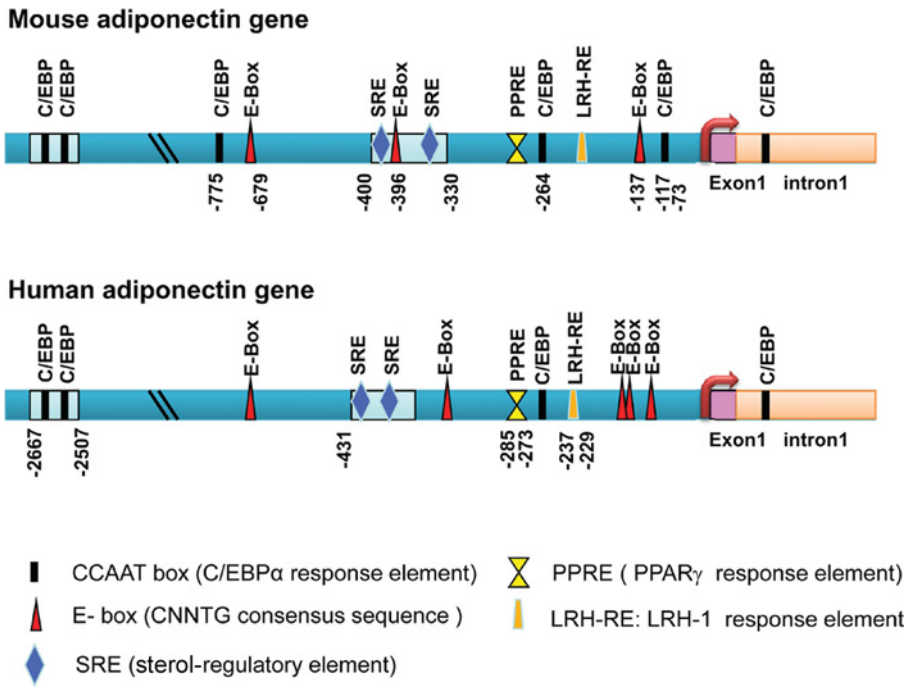

Figure 1 The basic structure of mouse and human adiponectin gene promoter

Two intronic and one distal C/EBP enhancers have been found [31,32]. Both mouse and human adiponectin gene promoters contain one PPRE, one liver receptor homologue 1-response element (LRH-RE), two SREs, several C/EBPα enhancer elements and a different number of the E-boxes.

chromosomal locus 3q27 [23]. The intron/exon structure, which is composed of three exons separated by two introns, is conserved between mouse and human adiponectin genes [23]. Deletion analysis of the human adiponectin promoter showed that the promoter region spanning from −676 to +41 is sufficient for basal transcriptional activity [24]. An approx. 1 kb fragment located upstream of the transcriptional start site of the mouse adiponectin gene has also conferred basal transcriptional activity [23,25]. Both the mouse and human adiponectin promoter lack a TATA-box, a feature that has been found in many other developmentally and differentially regulated genes [26]. On the other hand, the proximal 1 kb of the mouse adiponectin promoter contains three E-boxes containing the consensus sequence CANNTG (where N is any nucleotide), which are recognized by class I bHLH (basic helix–loop–helix) proteins (E-proteins) [27]. The adiponectin gene also contains a PPRE (PPAR-responsive element) [28], a classical CCAAT box [23,25,29], several C/EBPα enhancers [30–32], and a putative SRE (sterol-regulatory element) [24] (Figure 1). The presence of multiple transcription-factor-binding sites suggests that the transcription of the adiponectin gene is subject to an elaborate regulation by diverse upstream signals.

Transcription factors promoting adiponectin gene transcription

PPARγ

PPARγ is a member of the PPAR subfamily of transcription factors that also include PPARα and PPARβ/δ. A large body of evidence confirms that PPARγ, which is expressed mainly in adipose tissue, is a positive regulator of adiponectin gene expression. Adipose-tissue-specific deletion of the PPARγ gene decreased plasma adiponectin levels in mice [33]. In addition, P12A mutation, which has been found to disrupt the transcriptional activity of PPARγ [34,35], significantly correlates with reduced serum adiponectin level in the Japanese population [36], although there are some data suggesting that ethnic background and/or environmental factors may contribute to PPARγ-mediated regulation of adiponectin gene expression [37]. Adiponectin transcription is stimulated by the PPARγ agonists TZDs, both *in vitro* and *in vivo* [28,38,39]. In addition, both the human [28] and mouse [40] adiponectin promoters contain a putative PPARγ-recognizing PPRE site, and point mutations at this site lead to reduced basal and TZD-induced adiponectin promoter transactivation [28] (Figure 1). Together, these findings suggest that PPARγ may enhance cellular adiponectin levels by acting directly on its promoter. However, some studies have shown that treating adipocytes with TZDs had no effect on the mRNA levels of adiponectin, but have stimulated the biosynthesis and secretion of the HMW form of this adipokine [41,42], suggesting that the stimulatory effect of TZDs may occur primarily at the level of translation and/or post-translational modification.

FoxO1

FoxO1 is a member of the forkhead box O transcription factor family involved in the regulation of adipocyte differentiation [43]. The cellular localization and activity of FoxO1 is regulated by post-translational modification such as phosphorylation and acetylation. Three predicted PKB (protein kinase B)/Akt phosphorylation sites (Thr^{24}, Ser^{256} and Ser^{319}) have been identified in FoxO1, and phosphorylation of Ser^{256} is critical for insulin and IGF-1 (insulin-like growth factor 1)-induced suppression of FoxO1 activity and function [44]. The function of FoxO1 is also regulated by the NAD-dependent deacetylase Sirt1 (sirtuin 1), which deacetylates three lysine residues within the DNA-binding domain of FoxO1 and promotes FoxO1 nuclear translocation [45,46] (Figure 2). A number of studies suggest that FoxO1 positively regulates adiponectin transcription. First, FoxO1 haploinsufficiency decreases adiponectin levels [43]. In addition, overexpression of wild-type FoxO1 or mutants of

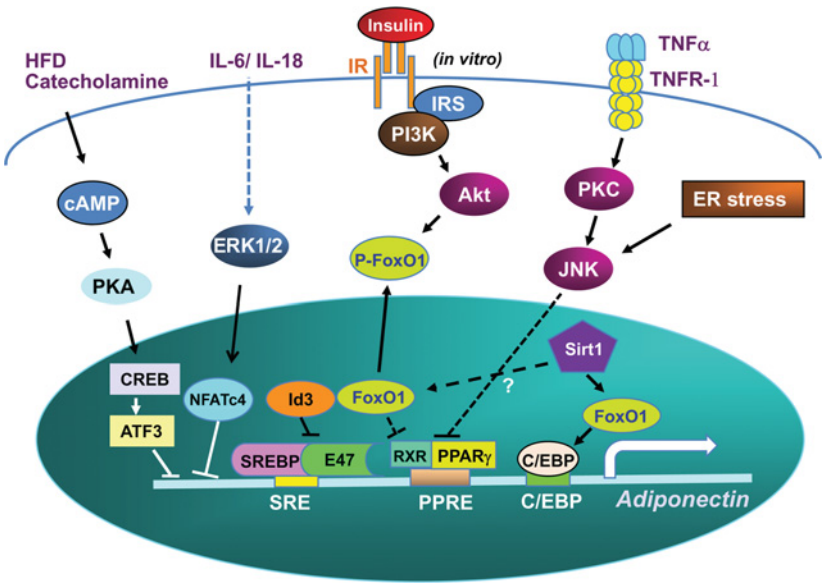

Figure 2 Regulation of adiponectin transcription by upstream signals

In obesity, increased pro-inflammatory cytokines such as TNFα, IL-6 and IL-18 negatively regulate adiponectin gene transcription by activating several pathways such as the JNK and ERK1/2 pathways. High-fat-diet (HFD)-induced obesity also suppresses adiponectin expression by increasing cellular levels of catecholamine and PKA-mediated activation of CREB [85]. Insulin has been suggested to positively regulate adiponectin gene expression by activating PPARγ via suppressing FoxO1 activity *in vitro* [50]; however, a negatively correlated relationship has been documented between insulin and adiponectin levels *in vivo* (see the text). FoxO1 could have a positive effect on adiponectin transcription via interaction with C/EBP [49,169]. Regulation of FoxO1 by insulin and Sirt1 may provide a mechanism to dynamically regulate adiponectin gene expression. IR, insulin receptor; IRS, insulin receptor substrate; P-FoxO1, phosphorylated FoxO1; PI3K, phosphoinositide 3-kinase; RXR, retinoid X receptor; TNFR-1, TNFα receptor 1. An animated version of this Figure can be seen at http://www.BiochemJ.org/bj/425/0041/bj4250041add.htm

FoxO1 that either mimic Sirt1-mediated deacetylation or lack the Akt-phosphorylation site (Ser^{256}) increases adiponectin gene expression [47,48]. Furthermore, FoxO1 knockdown decreases adiponectin levels [47]. Consistent with the involvement of FoxO1 in the regulation of adiponectin gene expression, two FoxO1-responsive elements have been identified in the mouse adiponectin promoter [49]. FoxO1 forms a complex with C/EBPα, and formation of this complex is stimulated by overexpression of Sirt1, resulting in adiponectin promoter activation [49] (Figure 2). Taken together with the finding that the protein levels of FoxO1 and Sirt1 are greatly reduced in fat tissues from high-fat-diet-induced obese and Type 2 diabetic mouse models [49], these results suggest a potential mechanism underlying obesity-induced insulin resistance in obesity. However, FoxO1 has been found to suppress PPARγ gene expression [50]. Since PPARγ positively regulates adiponectin gene expression and secretion, these findings suggest that the effects of FoxO1 on adiponectin biosynthesis may depend on cell content and upstream signal events.

C/EBPα

C/EBPα belongs to a family of transcription factors composed of six members (α–ζ). C/EBPα undergoes homo- or hetero-dimerization necessary for interaction with the CCAAT box motif and recruitment of co-activators such as CBP [CREB (cAMP-response-element-binding protein)-binding protein] that, in turn, regulates the transcription of many target genes [51]. There is some evidence suggesting that adiponectin gene transcription is stimulated by C/EBPα. In C/EBPα-deficient adipocytes, ectopic expression of PPARγ only modestly increased the levels of adiponectin, but adiponectin expression levels could be markedly enhanced by co-expression of both PPARγ and C/EBPα [52,53]. These findings suggest that C/EBPα is required to fully activate adiponectin gene transcription. Consistent with a stimulatory role of C/EBPα in adiponectin gene transcription, overexpression of C/EBPα or suppression of its expression by siRNA (short interfering RNA) significantly increased or decreased respectively, adiponectin mRNA levels in differentiated human adipocytes [32]. Two C/EBPα enhancers have been identified in the human adiponectin promoter [30,31]. In addition to these enhancers, a 34-bp core sequence for an intronic enhancer, which contains three tandem C/EBP-responsive elements, has also been identified in the human adiponectin gene [32]. Although this intronic enhancer is not conserved between humans and mice, ectopic expression of C/EBPα increased adiponectin mRNA levels in both human and mouse adipocytes, suggesting that C/EBP up-regulates adiponectin gene expression, perhaps through a mechanism that is conserved between humans and mice [32] (Figure 2). However, some reports showed that C/EBPα has no effect on adiponectin promoter activity [23,40,52,54]. In addition, it has been shown that C/EBPα expression is not different in adipose tissues between subjects with insulin resistance/the metabolic syndrome and controls [55–57]. Furthermore, the expression of C/EBPα in subjects with the metabolic syndrome is reduced by caloric restriction, which has been shown to increase the cellular levels of adiponectin in rats [58], although the same study showed that C/EBPα negatively correlates with BMI (body mass index), waist–hip ratio and plasma glucose levels [59]. Thus the physiological significance of C/EBPα in regulating adiponectin expression *in vivo* remains to be clarified further.

SREBPs

SREBPs belong to the bHLH leucine zipper class of transcription factors that bind to the SRE DNA sequence TCACNCCAC [60]. SREBPs are synthesized as membrane-bound precursors which interact with the nuclear envelope or ER (endoplasmic reticulum) membranes. In response to cues induced by cholesterol depletion,

SREBP is proteolysed to an active and water-soluble N-terminal domain which is subsequently translocated into the nucleus where it interacts with the SREs of the target genes. The binding of SREBP to SREs promotes transcription and up-regulation of enzymes involved in lipid metabolism [61–63].

A putative SREBP-responsive element required for basal promoter activity has been identified in the human adiponectin promoter [24]. In addition, the adiponectin promoter is transactivated by SREBP-1c and the activation is abolished by mutations in the SRE motif [40]. Furthermore, adenoviral overexpression of SREBP-1c increased adiponectin mRNA and protein levels in 3T3-L1 adipocytes. SREBP-1c also promotes adiponectin transcription by association with another bHLH factor E47 and subsequent binding to E-boxes within the adiponectin promoter [64]. Taken together, these results suggest that SREBP-1c plays a stimulatory role in adiponectin gene transcription (Figure 2). However, a critical question that remains to be answered is whether activation of the adiponectin gene by SREBP-1c in adipocytes is physiologically relevant *in vivo*. Increased levels of SREBP-1c in liver are associated with insulin resistance and fatty livers in mouse models of diabetes [65,66]. In addition, overexpression of an active form of SREBP-1c in hepatocytes leads to steatosis [67–69]. Furthermore, reducing ER-stress-induced SREBP-1c proteolytic cleavage improves steatosis and insulin sensitivity of *ob*/*ob* mice [70]. One possible explanation for these discrepancies could be that SREBP-1c plays a distinct role in hepatocytes and adipocytes. Whereas activation of this transcription factor in the liver leads to insulin resistance and steatosis, activation of this gene in adipocytes may improve insulin sensitivity by activating the adiponectin gene. Further studies will be needed to test these possibilities.

Repressors of adiponectin gene transcription in obesity

It is well documented that serum adiponectin levels are negatively correlated with obese in both animals and humans [2,71–74]. However, the precise underlying mechanism remains unclear. In obesity, increased fat mass results in a hypoxic microenvironment [75–77], which has been shown to suppress adiponectin expression via the HIF-1α (hypoxia-induced factor-1)-dependent pathway [78]. Obesity also leads to a low-grade chronic inflammatory state accompanied by increased production of pro-inflammatory cytokines such as TNFα, IL-6 and IL-18 and reduced adiponectin gene expression [79–82]. Several transcription factors, such as CREB [83–85] and NFAT (nuclear factor of activated T-cells) [86,87], have been identified as repressors that contribute to obesity-induced down-regulation of adiponectin gene transcription.

CREB

During fasting, CREB prompts expression of the gluconeogenic genes in liver to regulate glucose homoeostasis [88]. Constitutively stimulated hepatic CREB activity contributes to hyperglycaemia and insulin resistance in diabetes [83,84]. CREB also mediates the effects of catecholamines to enhance lipolysis in WAT (white adipose tissue) and has been identified as a key regulator of adipogenesis [89,90]. Enhanced CREB activity has been linked to systemic insulin resistance in obesity [91]. Recent studies indicate that CREB acts as a transcriptional repressor of adiponectin gene expression [85]. Adipose-tissue-specific expression of a dominant-negative CREB transgene increased the levels of adiponectin mRNA and circulating HMW adiponectin protein compared with wild-type controls, but had no effect on plasma concentrations of resistin, RBP4 (retinol-binding protein 4), TNFα and IL-1β [85]. However, CREB does not bind directly to the adiponectin promoter, but instead inhibits adiponectin transcription by up-regulation of the transcriptional repressor ATF3 (activating transcription factor 3) [85] (Figure 2), a transcriptional factor that has been shown previously to binds to the putative AP-1 (activator protein-1) site located adjacent to the NFAT-binding site to repress adiponectin promoter activity [92].

NFAT

NFAT is a family of transcription factors that contains four members, NFATc1–NFATc4. Although the calcium-sensitive NFATc1, NFATc2 and NFATc3 have been shown to be tightly restricted to the immune system, NFATc4 appears to have a more broad function [93]. NFAT proteins have been detected in 3T3-L1 adipocytes [86] and mouse adipose tissues [92]. In addition, the binding activities of these transcriptional factors are significantly increased in WATs of *ob*/*ob* and *db*/*db* mice compared with controls, consistent with a negative role of these transcriptional factors in adiponectin expression in obesity and Type 2 diabetes [92]. Deletion of the putative NFAT-binding site in mouse adiponectin promoter increased the promoter activity, and overexpression of NFATc4 reduced adiponectin promoter activity [92] (Figure 2), suggesting that endogenous NFAT is a negative regulator of adiponectin transcription. However, how activation of NFAT leads adiponectin gene suppression remains to be elucidated.

In addition to CREB and NFAT, several other transcription factors, including activation of the AP-2β (activating enhancer-binding protein-2β), IGFBP-3 (IGF-1-binding protein 3) and Id3 (inhibitor of differentiation-3), have also been implicated in the negative regulation of adiponectin gene expression. AP-2β inhibits adiponectin transcription directly by displacing NF-Y (nuclear factor-Y) [94]. IGFBP-3 binds to RXRα (retinoid X receptor α) to suppress its dimerization with PPARγ that is necessary for adiponectin gene transcription [78]. Id3 interacts with the SREBP-1c co-activator E47 and prevents E47 binding to E-boxes within the adiponectin promoter [64] (Figure 2). Adiponectin mRNA expression is also suppressed via β-adrenergic signalling-mediated activation of PKA (protein kinase A) [95,96], which has long been known to induce insulin resistance [97,98].

Regulation of adiponectin transcription by inflammatory cytokines

Obesity-induced macrophage filtration into adipocytes and low-grade inflammation have been implicated in many clinically important complications such as insulin resistance, diabetes, atherosclerosis, non-alcoholic fatty liver disease and cardiovascular disease [99]. In obesity, the enhanced production of pro-inflammatory cytokines such as TNFα and ILs is well documented to suppress adiponectin expression in adipocytes [78,81,100,101].

TNFα

Several mechanisms have been proposed to depict TNFα-induced down-regulation of adiponectin gene expression in adipocytes. TNFα suppresses the expression levels of activators involved in promoting adiponectin gene expression, such as PPARγ [102], C/EBP [24,103] and SREBP [49,104]. The suppressive effect of TNFα on adiponectin transcription may be mediated by JNK (c-Jun N-terminal kinase), which has been shown to phosphorylate PPARγ and decrease its DNA-binding activity [100,105] (Figure 2). It has been shown that the activation of JNK activity induced by TNFα is PKC (protein kinase C)-

dependent [100] (Figure 2). TNFα has also been suggested to suppress adiponectin gene transcription by inhibiting the transcriptional Sp1-binding activity [54]. Furthermore, TNFα promotes the expression of IGFBP-3, which suppresses adiponectin transcription and induces insulin resistance [78].

IL-6

IL-6 is an adipokine that exerts both pro-inflammatory and anti-inflammatory functions [106,107]. The serum concentrations of IL-6 are elevated in insulin-resistant states such as obesity [108–110], impaired glucose tolerance [111] and Type 2 diabetes [112,113]. IL-6 treatment suppressed adiponectin mRNA levels and its secretion in 3T3-L1 adipocytes, and this inhibition was partially reversed by pre-treatment of cells with pharmacological inhibitors of a p44/42 MAPK (mitogen-activated protein kinase) [96] (Figure 2), suggesting a negative role of IL-6 in the modulation of adiponectin levels and insulin sensitivity. However, incubation of human adipose tissue fragments with IL-6 had no effect on adiponectin mRNA levels [82], suggesting that 3T3-L1 cells may have different characteristics compared with human adipose tissue. Further studies should clarify whether IL-6 negatively regulates adiponectin gene expression *in vivo*.

IL-18

IL-18 levels are increased in obesity and Type 2 diabetes [114–116] and are inversely correlated with the plasma levels of adiponectin [117,118]. Treating 3T3-L1 adipocytes with IL-18 selectively suppressed the expression levels of adiponectin [101]. IL-18 induces ERK (extracellular-signal-regulated kinase) 1/2-dependent phosphorylation and activation of NFATc4, which has been shown previously to function as a repressor in adiponectin transcription [92] (Figure 2). The inhibitory effect of IL-18 on adiponectin promoter activity was diminished by inactivation of ERK1/2 or RNAi (RNA interference)-mediated suppression of NFATc4 [101]. Together, these results suggest that IL-18 may have a causal effect on obesity-induced suppression of adiponectin gene expression.

REGULATION OF ADIPONECTIN BIOSYNTHESIS AND SECRETION BY POST-TRANSLATIONAL MODIFICATION

Adiponectin is synthesized as a single polypeptide of 30 kDa and is then assembled in the ER into trimeric, hexameric and HMW multimeric forms [14–16,41]. Different adiponectin multimers have been shown to exert distinct biological functions [8,119,120]. The trimeric and hexameric forms of adiponectin play a major role in the brain to regulate food intake [13,121]. The HMW form of adiponectin, on the other hand, has been found to be more metabolically active and more closely associated with peripheral insulin sensitivity than the trimeric and hexameric forms of this adipokine [122,123]. Adiponectin mutants with impaired multimerization are defective in both secretion and function, and are associated with diabetes and hypoadiponectinaemia [14,16]. More importantly, it has been shown that adiponectin oligomer distribution, rather than its absolute levels, correlates with TZD-mediated increase in insulin sensitivity [19].

Regulation of adiponectin multimerization and secretion by hydroxylation and glycosylation

The mechanism and roles of adiponectin hydroxylation and glycosylation have been reviewed extensively recently [20]. In the present review, we briefly highlight the structural feature of adiponectin hydroxylation and glycosylation and the clinical relevance of these modifications. The full-length adiponectin protein consists of a signal sequence at the N-terminus, followed by a variable region, a collagenous domain and a C-terminal globular domain. There are four conserved lysine residues in the collagenous domain of adiponectin (Lys^{65}, Lys^{68}, Lys^{77} and Lys^{101} for human adiponectin) which are modified by hydroxylation and subsequent glycosylation [124,125]. The hydroxylation and glycosylation are required for intracellular assembly of the trimer of adiponectin into the HMW multimer and dysregulation in the post-translational modification process impairs adiponectin stability and secretion [20,124,125]. In addition to these lysine residues, hydroxylation is also detected at several proline residues in human adiponectin, including Pro^{71}, Pro^{76} and Pro^{95}. Although inhibition of proline hydroxylation resulted in a more severe impairment of adiponectin multimerization, the physiological role of this hydroxylation remains to be clarified further [125].

Regulation of adiponectin multimerization and secretion by disulfide bond formation

The formation of intermolecular disulfide bonds between Cys^{36} in human (Cys^{39} in mouse) adiponectin is essential for adiponectin multimerization and secretion. The C39S mutation completely disrupted the assembly and secretion of hexameric and HMW adiponectin, but had very little effect on trimer formation [16]. Two studies have previously demonstrated that Ero1 (ER membrane-associated oxidoreductase)-Lα and its associated protein ERp44 play an important role in the assembly of higher-order adiponectin complexes and secretion [41,126] (Figure 3). ERp44 retains adiponectin in the ER by interacting covalently with the thiol group of Cys^{39} of this adipokine. The thiol-mediated retention maintains adiponectin in an oxidizing environment for post-translational modification such as disulfide bond formation. Suppression of Ero1-Lα by RNAi reduces adiponectin secretion during the differentiation of 3T3-L1 preadipocytes. On the other hand, ectopic expression of Ero1-Lα in Ero1-Lα-deficient 3T3 fibroblasts stimulates adiponectin secretion following the conversion of the cells into adipocytes [41]. Ero1-Lα promotes adiponectin release by competing with ERp44 to bind to adiponectin, which facilitates HMW adiponectin formation and secretion [126].

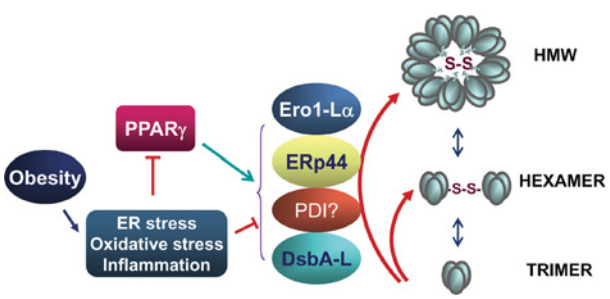

Figure 3 Potential mechanisms regulating adiponectin multimerization

PPARγ may stimulate adiponectin multimerization by enhancing the expression levels of ER proteins such as Ero1-Lα, DsbA-L and an uncharacterized PDI(s). The expression levels of Ero1-Lα, DsbA-L and the putative adiponectin-specific PDI are negatively regulated by obesity-induced pro-inflammatory cytokines such as TNFα. The oxidized PDI catalyses the formation of intermolecular disulfide bonds to facilitate adiponectin folding into their stable conformation and multimerization. Ero1-Lα may function as an oxidase of an adiponectin-specific PDI to promote sustaining disulfide-linked adiponectin folding in the ER. DsbA-L may interact with an adiponectin-specific PDI *in vivo* to facilitate adiponectin assembly and secretion.

Another mechanism by which Ero1-Lα regulates adiponectin multimerization is by functioning as an oxidoreductase. Both Ero1-Lα and its alternative isoform Ero1-Lβ, which differ in their structure, regulation and tissue distribution [127], are able to catalyse protein disulfide bond formation in mammalian cells [128]. In the ER, these oxidoreductases utilize the oxidizing power of oxygen to generate disulfide bonds in themselves and then undergo dithiol–disulfide exchange with PDIs (protein disulfide-isomerases). The oxidized PDI then catalyses the formation of intra- and inter-molecular disulfide bonds to facilitate protein folding into their stable conformation [129]. Thus Ero1s function as oxidoreductase of PDI responsible for sustaining disulfide-linked protein folding in the ER. So far, a total of 19 PDI family members have been identified in the human ER, and available evidence suggests that individual PDIs have distinct substrates [130,131]. It is possible that Ero1-Lα may oxidize a distinct PDI to specifically regulate adiponectin multimer formation. In addition to Ero1-Lα and ERp44, GGA [Golgi-associated γ-adaptin ear homology domain Arf (ADP-ribosylation factor)-interacting protein] has also been implicated in adiponectin trafficking and secretion, although the protein does not interact directly with adiponectin [132].

Regulation of adiponectin multimerization and secretion by DsbA-L

DsbA-L (disulfide-bond A oxidoreductase-like protein), previously named GST (glutathione transferase) Kappa, is a 25-kDa adiponectin-interactive protein identified recently through a yeast two-hybrid cDNA library screening using mouse adiponectin as bait [22]. Analysis of the complete amino acid sequence of DsbA-L reveals that this protein has very little sequence similarity to any other class of GST and exhibits no significant transferase activity for physiologically relevant GST substrates [133]. In addition, this protein lacks the SNAIL/TRAIL (Ser-Asn-Ala-Ile-Leu/Thr-Arg-Ala-Ile-Leu) motif found in all other classes of soluble GSTs and does not bind to agarose-coupled glutathione [134]. On the other hand, the protein contains a non-catalytic b-type thioredoxin-like domain found in some PDIs, presumably participating in peptide-binding and/or chaperone-like activities [135–137]. On the basis of these findings, GST Kappa has been acknowledged to be misnamed in the protein sequence database and not a member of the GST gene family [138].

DsbA-L is expressed in various mouse tissues such as liver, kidney, pancreas and heart, but the highest expression of this protein is detected in adipose tissue, where adiponectin is synthesized and secreted [22]. The cellular levels of DsbA-L are significantly reduced in adipose tissues of obese mice and human subjects. Like adiponectin, DsbA-L expression in 3T3-L1 adipocytes is stimulated by the insulin sensitizer rosiglitazone and inhibited by the inflammatory cytokine TNFα (Figure 3). Overexpression of DsbA-L promotes adiponectin multimerization while suppressing DsbA-L expression by RNAi markedly and selectively reduces adiponectin levels and secretion in 3T3-L1 adipocytes. Altering the cellular levels of DsbA-L has no effect on the cellular levels of several other adipokines such as leptin, resistin and TNFα, suggesting a selective role for this protein in regulating adiponectin assembly and secretion [22] (Figure 3). Taken together, these findings suggest that DsbA-L may be a potential therapeutic target for improving insulin sensitivity. However, a number of questions remain to be addressed further. Does DsbA-L function as a protein-folding catalyst or a molecular chaperone in facilitating adiponectin assembly? Does DsbA-L exert its role in facilitating adiponectin multimerization by interacting with other ER proteins such as a PDI? Does DsbA-L regulate the post-translational modification and function of other cellular proteins? What are the functional roles of DsbA-L *in vivo*? Further investigations will be needed to answer these important questions.

Negative regulation of adiponectin biosynthesis and secretion by ER stress, oxidative stress and pro-inflammatory cytokines

Obesity leads to ER stress [139], which has been linked to the inhibition of adiponectin production in adipose tissue. Adiponectin mRNA expression in adipose tissue of obese mice was negatively correlated with the expression levels of CHOP (C/EBP homologous protein), an ER stress marker [79]. In cultured adipocytes, impairment in mitochondrial function, which induces ER stress and activation of JNK and ATF3, inhibits adiponectin expression [140] (Figure 2). On the other hand, reducing ER stress and inactivation of JNK by the PPARα/γ dual agonist macelignan increases adiponectin expression in adipose tissue of *db*/*db* mice [141]. The expression levels of adiponectin are also negatively correlated with oxidative stress and inflammation [142,143]. Treating 3T3-L1 adipocytes with H_2O_2 or glucose oxidase and 4-HNE (4-hydroxynonenal), a relevant lipid peroxidation by-product which makes covalent adducts with various molecules, greatly decreased adiponectin secretion [143,144]. Adiponectin expression levels and secretion in 3T3-L1 adipocytes are also suppressed by inflammatory cytokines such as TNFα or IL-6 [96,100]. The mechanisms underlying obesity-induced adiponectin down-regulation remain largely elusive, but a recent study reveals that the inhibitory effect of H_2O_2 on adiponectin expression and secretion could be mediated by Akt and JAK (Janus kinase)/STAT (signal transducer and activator of transcription) signalling pathways [145]. In addition, activation of the conventional PKC isoforms and the JNK signalling pathway have been implicated in the inhibitory effect of TNFα [100,105]. Another possible mechanism underlying obesity-induced inhibition of adiponectin assembly and secretion may be due to suppression of PPARγ (Figure 3). Consistent with this view, the expression and/or activity of PPARγ is suppressed by an ER stress inducer [146] or TNFα [102,147]. Since PPARγ plays a critical role in the biosynthesis of enzymes/ER chaperones involved in adiponectin assembly and secretion such as Ero1-Lα and DsbA-L [22,41,126], suppressing its expression levels and/or activity could thus result in reduced adiponectin cellular levels and secretion.

Negative regulation of adiponectin secretion by testosterone

Circulating adiponectin concentrations are significantly higher in women than in men, and this sexual dimorphism has been attributed to an inhibitory effect of testosterone on adiponectin secretion from adipose tissue. In support of this, testosterone has been found to significantly reduce adiponectin serum levels in transsexual female patients [148] and in healthy [149], hypogonadal [150] and Type 2 diabetic [151] men. In addition, the total serum adiponectin levels and the ratio of HMW isomers to total adiponectin decrease through puberty in male Danish school children [152], suggesting that testosterone has a selective effect on the serum levels of the HMW form of adiponectin, which has been shown to be more closely associated with peripheral insulin sensitivity than the trimeric and hexameric forms of this adipokine [122,123]. In agreement with these findings, testosterone treatment specifically reduced, and castration dramatically increased, the HMW form of serum adiponectin in mice [153]. Interestingly, neither castration nor testosterone treatment had obvious effects on the intracellular pattern of adiponectin oligomeric complex distribution in fat tissue, suggesting

testosterone inhibits adiponectin secretion. Since the plasma testosterone levels are known to inversely correlate with insulin sensitivity in men, the selective inhibition of HMW adiponectin by testosterone could thus provide an explanation for why men usually have a higher risk of insulin resistance than women.

Whereas it is well established that testosterone has a negative effect on the serum levels of adiponectin, an important question not fully addressed is whether the inhibitory effect is direct or indirect. Xu et al. [153] showed that testosterone directly and selectively reduces the HMW form of serum adiponectin by inhibiting its secretion from rat adipocytes. On the other hand, Horenburg et al. [154] found that increasing concentrations of testosterone or oestradiol had no effect on either adiponectin mRNA expression and secretion or adiponectin multimerization in human fat cells. On the basis of these findings, it was hypothesized that there is a serum factor that is differently regulated by sex steroids and subsequently causes the sex dimorphism in circulating adiponectin levels. Further studies will be needed to clarify these controversies.

Insulin: negative or positive regulator of adiponectin biosynthesis and secretion?

The relationship between plasma insulin and adiponectin levels has been studied extensively, but the role of insulin in adiponectin biosynthesis and secretion remains controversial. In adipocytes, most, but not all [155], studies reported that insulin has a direct stimulatory effect on adiponectin gene expression [40,156–159]. Pulse–chase experiments have demonstrated that secretion of adiponectin, but not α3(VI) collagen, is enhanced by insulin in 3T3-L1 cells, suggesting that insulin selectively regulates adiponectin secretion [160]. Consistent with this view, treating 3T3-L1 adipocytes with insulin led to increased adiponectin secretion into the culture medium, but had no effect on the intracellular mRNA levels of adiponectin [161]. The stimulatory effect of insulin on adiponectin secretion is suppressed by inhibition of PI3K (phosphoinositide 3-kinase) and Akt, but not of mTOR (mammalian target of rapamycin) or MAPK [158,160,161]. The precise mechanism by which insulin stimulates adiponectin biosynthesis remains elusive, but suppressing the activity of FoxO1, a transrepressor of PPARγ, may contribute to the stimulation [50]. However, a negatively correlated relationship has been documented between insulin and adiponectin levels *in vivo*. Serum adiponectin levels are much higher in Type 1 diabetic patients [162] or in patients with genetically defective insulin receptors [163] compared with their healthy controls. In addition, hyperinsulinaemia significantly decreased plasma adiponectin levels under euglycaemic conditions [164]. There is also a report showing that the HMW form of adiponectin is selectively down-regulated in hyperinsulinaemia and Type 2 diabetes [165]. Furthermore, adipose-tissue-specific deletion of the insulin receptor gene led to increased plasma adiponectin levels [166]. Taken together, these findings suggest that insulin functions as a negative regulator of adiponectin biosynthesis and/or secretion *in vivo*. The reason that insulin has a different effect on adiponectin levels *in vitro* and *in vivo* remains unknown, but it is possible that insulin may activate some signalling pathways that indirectly suppress adiponectin biosynthesis and secretion *in vivo*. Further investigations should yield insights into this possibility.

Regulation of adiponectin biosynthesis and secretion by PPARγ

PPARγ has been identified as a regulator to promote adiponectin biosynthesis throughout the whole process, but some recent studies suggest that the primary effect of PPARγ may be to promote adiponectin assembly and secretion, rather than transcription. Consistent with this notion, pioglitazone treatment increased plasma levels of adiponectin in human subjects, but had no effect on adiponectin expression in adipose tissue [167]. In addition, treating adipocytes with PPARγ agonists increased the synthesis and secretion of HMW adiponectin without affecting mRNA expression and protein synthesis of this adipokine [41,42]. A likely mechanism by which TZDs increase adiponectin levels and secretion is to stimulate the expression of proteins involved in adiponectin assembly and secretion. In agreement with this, activation of PPARγ enhances the expression levels of Ero1-Lα in mature adipocytes and in mice [41,126]. Activation of PPARγ also increases cellular levels of DsbA-L, a versatile player in adiponectin multimerization and secretion [21,22] (Figure 3).

Regulation of adiponectin biosynthesis and secretion by Sirt1

Sirt1, the mammalian homologue of SIR2, regulates glucose and lipid metabolism through deacetylating various cellular substrates. Several studies suggest that Sirt1 has a prominent role in adiponectin biosynthesis and secretion in adipocytes. Both the expression levels of Sirt1 [46] and adiponectin [58] are up-regulated by caloric restriction. In addition, moderate overexpression of Sirt1 in mice increased adiponectin levels [47]. Furthermore, activation of Sirt1 by resveratrol increases adiponectin levels in obese Zucker rats [168] and prevents TNFα-induced suppression of adiponectin mRNA expression and secretion in 3T3-L1 adipocytes [169]. One potential mechanism underlying Sirt1-induced adiponectin up-regulation is probably through deacetylation of FoxO1, which traps FoxO1 in the nucleus to promote adiponectin gene expression [49,170]. However, Sirt1 has been found to suppress PPARγ activity in response to caloric restriction in starved animals and in 3T3-L1 adipocytes [171] (Figure 2). Since PPARγ is well documented to positively regulate adiponectin biosynthesis and secretion, this finding suggests that Sirt1 may be a negative regulator of adiponectin production and secretion. Consistent with this notion, activation of Sirt1 by resveratrol has been shown to down-regulate the PPARγ-responsive gene Ero1-Lα, leading to suppression of HMW adiponectin secretion, whereas suppressing Sirt1 increases secretion of HMW adiponectin [41].

Up-regulation of adiponectin multimerization and secretion: a potential clinical therapy for insulin resistance and related metabolic disorders?

Although the beneficial effects of adiponectin on regulating energy homoeostasis and metabolism have been well documented, the development of adiponectin as a therapeutic drug for insulin resistance has been difficult. A major obstacle in this objective is the high plasma levels of the endogenous adiponectin, which makes further elevation of this adipokine *in vivo* rather challenging. In addition, endogenous adiponectin undergoes various post-translational modifications and multimerization, which appear to be critical for its biological function [8,19,119,120,172]. Thus it would be important that the recombinant adiponectin shares the same physiological properties as the native adiponectin multimers. The recent finding that adiponectin biosynthesis, multimerization and secretion are stimulated by the ER-associated proteins Ero1-Lα and DsbA-L [22,41] has shed some promising light on the development of adiponectin-related therapeutic interventions. Both Ero1-Lα and DsbA-L are targets of PPARγ, suggesting a common mechanism of regulation. In addition, overexpression of either Ero1-Lα or DsbA-L increases HMW adiponectin production and secretion in adipocytes [22,41,126].

Thus specific up-regulation of the expression levels of these ER proteins could be an effective approach to enhance adiponectin multimerization and secretion. It is entirely possible that one or more adiponectin-specific ER proteins may exist in cells that specifically regulate adiponectin assembly and secretion. Identification and characterization of these novel proteins should yield new insights into developing effective therapeutics to combat insulin resistance and related metabolic disorders.

CONCLUSION

Adiponectin exists in cells and in serum mainly as trimeric, hexameric and HMW forms, and defects in adiponectin multimerization impair adiponectin stability and secretion, and are correlated with insulin resistance *in vivo*. Adiponectin gene transcription is stimulated by several transcription factors involved in adipogenesis such as PPARγ, FoxO1, C/EBPα and SREBP, and is suppressed by hypoxia, inflammation, transcription repressors such as NFATs and CREB, and pro-inflammatory factors such as TNFα, IL-6 and IL-18 [78,101,173]. The stability and secretion of adiponectin are also regulated at the post-translational modification level via hydroxylation, glycosylation and disulfide bond formation. Impaired multimerization of adiponectin is associated with reduced plasma levels of adiponectin, obesity and insulin resistance. Several ER-associated proteins such as Ero1-Lα, ERp44 and DsbA-L have been identified that play critical roles in regulating adiponectin multimerization and/or secretion and are potential therapeutic drug targets for improving insulin sensitivity and action. Elucidation of the mechanisms regulating adiponectin multimerization and identification of the additional regulators in adiponectin biosynthesis and multimerization should uncover promising therapeutic targets for the diagnosis and treatment of insulin resistance and its associated diseases.

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